REGENERATION AND PLOIDY LEVEL OF PLANTS FROM ANTHER CULTURES OF WINTER RAPESEED

Angela Nałęczyńska, Jan Krzymański

Plant Breeding and Acclimatization Institute
Gorzów Wlkp., Poznań, Poland

Anther culture permits the isolation of homozygous lines from a segregating generation within a short time. A number of researches have recently reported on the use of this technique in several plant species including Brassica as well /Keller, Armstrong and de la Roche, 1983/. The majority of studies with Brassica napus have been conducted on spring rather than winter varieties. In this report we present results of dihaploid lines production from winter F_1 hybrids of rapeseed which were of particular interest in our breeding programme.

Materials and methods

Plants derived from F_1 seeds of two winter hybrids denoted M-1 and M-2, were grown in a natural environment in the field. Anthers were collected during a few days of rapeseed blooming time. Buds in which petal lenght varies from one third to two third of anther lenght, were sterilised in 70% ethanol for 10 mins and rinsed once with sterile distilled water. In the first phase the anthers were planted on the B5 medium developed by Gamborg et al., 1968 and modified by Keller and Armstrong, 1977.
Cultured anthers were incubated in darkness at 35°C for 2 days before transfer to 25°C until the emergence of macroscopic embryos. Such structures were transferred to the same B5 medium with sucrose level reduced to 2% but without any hormones. The plants were regenerated either directly from the embryos on the B5 medium or more frequently, through secondary embryogenesis in hypocotyl explants. At this stage of work a Murashige and Skoog /MS/ medium with or without an addition of BAP and NAA was used.

Plantlets with well-developed root systems were planted into the soil and maintained in the greenhouse. Seedlings were vernalised by keeping them at 2-4°C for 6 weeks at 8-hours photoperiod.

The identification of ploidy level of haploids and diploids could be quickly screened in flowering populations. In contrast to the normal diploid flowers haploid flowers have narrower petals and shrunken anthers. Root tips chromosome counts were also utilized to determine ploidy level of some morphologically non-typical forms.

Doubling the chromosome of haploid plants was done through colchicine treatment. Two or three young secondary auxilliary shoots were cut off from each haploid plant and placed into 0.05% colchicine solution in 1.5% DMSO for 18 h at 25°C in the dark. Then the cuts were rinsed with water, planted to the soil and rooted. Using this technique fertile dihaploid plants and seeds could be obtained from unfertile haploid plants.

Results and discussion

Microscopic observations of rapeseed anthers showed that the division of the pollen grains occured as early as 48 to 72 hours after culturing. Embryo emergences were completed in 35 days. Table 1 summarizes the data of embryogenesis from anthers of hybrids M-1 and M-2. Out of 1200 cultured anthers 971 embryos ranging from globular to fully differentiated stage were obtained. In comparison with our previous work /Nałęczyńska and Cegielska, 1984/ anthers of rapeseed collected from the field had given higher embryo yield /average - 80.9%/ than those cultivated in greenhouse /average - 10.3%/. Considering the fact that at present too little is known about all factors affecting the plant development in artificial environment there is probability of better results obtained with donor plants derived from natural conditions. Like as in other Brassica species /Keller and Armstrong, 1983, Ockendon, 1985/ extreme variability in embryo yield was observed amongst anther plantings /Table 1/ which was probably due to genotypic

plant to plant differences.

Table 1. Embryo yield in anther culture of winter rapeseed

Genotype	No. anthers cultured	No. embryos	Embryo yield per 100 anthers	
			Average	Range
Hybrid M-1	528	559	105.9	2.8-312.5
Hybrid M-2	672	412	62.3	21.9-107.5

Approximately 10% of the embryos developed directly into plants. The remainder plants were obtained from the explants of secondary embryogenic tissues on MS medium. It was observed that whole plants were regenerated more easily on hormone-free medium than in the presence of BAP and NAA. A total number of regenerated plants and their ploidy level is given in Table 2.

Table 2. Ploidy of plants regenerated from microspore - derived embryos

Genotype	No. plants	Haploids %	Diploids %	Mixoploids[x] %
Hybrid M-1	349	32.7	64.2	3.1
Hybrid M-2	274	42.4	48.5	9.1
Total	623	37.6	56.3	6.1

x/ Mixoploids include few forms which ploidy level has not been established to date.

There were found the differences in the ploidy level between regenerates derived from the same microspore embryo by means of secondary embryogenesis. Such variability between the clones of single androgenetic line is difficult to explain, but appears to be a common feature because similar cases were also detected in dihaploid lines received previously from other genotypes of rapeseed.

/Nałęczyńska and Cegielska, in press/.

The preliminary observations of dihaploid lines growing in the field showed that only part of them could be used in subsequent breeding. Number of the best lines was dependent to a great extent on the potential of donor plant genotype.

Described procedure of anther culture can not be recommended as a technique which should be applied to direct breeding of new varieties but it seems to be very useful in the combination with classical plant breeding methods.

References

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